165. The pharmaceutical formulation of claim 163, wherein said antibody is a genetically-engineered monoclonal antibody.

166. The pharmaceutical formulation of claim 165, wherein said antibody is a single-chain antibody.

167. The pharmaceutical formulation of any one of claims 162-166, wherein said beta-amyloid is human beta-amyloid.

REMARKS

Claims 1-4 and 150-167 presently appear in this case. No claims have been allowed. The official action of August 22, 2003, has now been carefully studied. Reconsideration and allowance are hereby respectfully urged.

The following statements are made pursuant to the requirements of 37 C.F.R. §1.173(c). Patent claims 1-4 are pending. Added claims 5-149 have been cancelled. Claims 150-167 are newly presented in the present amendment. As for an explanation of the support in the disclosure of the patent for the changes made to the claims, reference is made to the attached chart entitled "Support for New Reissue Claims." This chart sets forth examples of support in the disclosure of the patent for each of the claim limitations.

Briefly, the present invention relates to pharmaceutical formulations comprising an antibody or an antigen binding fragment thereof and a pharmaceutically acceptable carrier. The antibody and fragment recognize an

epitope within residues 1-28 of β-amyloid or are obtainable using residues 1-28 of β-amyloid as an immunogen and they inhibit aggregation of β-amyloid or they maintain the solubility of soluble β-amyloid. The antibody is preferably a monoclonal antibody, and more preferably a human monoclonal antibody, a genetically engineered monoclonal antibody, or a single chain antibody. The β-amyloid is preferably human β-amyloid.

The interview among Examiner Nichols, S.P.E. Kunz, attorney Gordon Kit, and the undersigned on February 11, 2004, is hereby gratefully acknowledged. In this interview, the claims submitted herewith were discussed as was the data reported in the declaration of Prof. Beka Solomon attached hereto. Furthermore, the nature of the aggregation assay appearing in the specification was clarified for the examiner. The arguments presented at the interview will be substantially repeated in the discussion of the rejections below.

The official action of August 22, 2003 was a final rejection. Withdrawal of the finality of this rejection, however, is hereby respectfully urged.

MPEP 706.07(a) says:

> Under present practice, second or any subsequent actions on the merits shall be final, except where the examiner introduces a new ground of rejection that is neither necessitated by applicant's amendment of the claims, nor based on information submitted in an information disclosure statement filed during the period set forth in 37 C.F.R. 1.97(c) with the fee set forth in 37 C.F.R. 1.17(p).

In the Official action of August 22, 2003, claim 126 was rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement. Claim 126 is substantially the same as previously appearing claim 20. However, in the previous Official action on the merits of June 29, 2001, claim 20 was not made the subject of a 35 U.S.C. §112 rejection. It is apparent that the present rejection under 35 U.S.C. §112 would have been equally applicable to previously appearing claim 20, so it is clear that this rejection was not necessitated by applicant's amendment. Thus, in accordance with the present policy the Patent and Trademark Office as set forth in the above-quoted portion of the MPEP, the finality of this Official action was premature. Reconsideration and withdrawal thereof is respectfully urged.

It is noted that in the interview of February 11, 2004, the examiners agreed that the finality of the Official action of August 22, 2003, would be withdrawn.

On January 22, 2004, a notice of appeal was filed in this case. Regardless of the finality of the Official action of August 22, 2003, the notice of appeal is effective because this is at least the second Official action on the merits. In view of the withdrawal of the finality of the Official action of August 22, 2003, the present amendment should be entered as a matter of right and appropriately responded to. Thus, applicant has elected to continue prosecution, as is permissible in view of the withdrawal of the finality of the

previous Official action, rather than to continue with the appeal.

The examiner has objected to the specification because there is a typed correction next to the abstract as filed ("[molecules]") and then typed next to this is "involves" typed over something covered in whiteout. The examiner states that this correction or alteration has not been entered as an amendment.

While it is believed that this type of amendment to the abstract is in full accordance with 37 C.F.R. §1.173(b) and (d), nevertheless the amendment to the abstract is being re-presented by the present amendment. It is believed that this objection has now been obviated and the correction to the abstract may now be entered.

The examiner states that the original patent, or a statement as to loss or inaccessibility of the original patent, must be received before this reissue application can be allowed.

Submitted herewith is the original Letters Patent with respect to patent no. 5,688,651. Accordingly, the requirement of 37 C.F.R. §1.178 has been met and the present application can proceed to allowance.

Claims 1-4 and 126-149 have been rejected as being based upon a defective reissue declaration. The examiner states that a supplemental reissue declaration must be received before the reissue application can be allowed. The examiner states that receipt of an appropriate supplemental

declaration under 37 C.F.R. §1.175(b)(1) will overcome this rejection under 35 U.S.C. §251.

Attached hereto is a supplemental declaration under 37 C.F.R. §1.175(b)(1). Accordingly, this rejection has now been obviated. As this was the only rejection of claims 1-4, these claims should now be considered to be in condition for allowance.

In the Official action of August 22, 2003, the examiner stated that claims 126-149 were rejected under 35 U.S.C. §251 for lack of defect or error in the original patent, and as not being an error correctable by reissue. Pursuant to a telephone interview of August 25, 2003, an interview summary form was issued correcting this line of the Official action, and confirming that "in fact only claims 130-149 are rejected." The summary record goes on to state that the first line of paragraph 12, page 3 of the final rejection of August 22, 2003 should read, "Claims 130-149 are rejected under 35 U.S.C. §251..."

Claims 130-149 have now been deleted without prejudice toward the continuation of prosecution thereof in a continuing application. Accordingly, this rejection has now been obviated.

Claims 126-129 have been rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement. This rejection is respectfully traversed.

At paragraphs 18 and 19, the examiner questions whether the "denatured" CPA protein is in fact "aggregated," and questioned whether the data supports the claims. In this regard, it should be noted that the present claims do not cover monoclonal antibodies specific for CPA, as the present claims are all directed to antibodies and fragments thereof that recognize an epitope of β-amyloid. It should be noted for the record, however, that it is aggregation, which causes the CPA protein to become denatured. Note the present specification at column 9, lines 49-52 and 57; column 10, line 52; column 12 lines 53-57; and column 13 line 53.

In paragraph 20 of the Official action, the examiner contends that there is no evidence in the Solomon application or Solomon (PNAS 1996)[1] of prevention of Aβ aggregates because the assay includes the step of removing aggregates.

The examiner appears to have misunderstood the assay technique employed. More specifically, in the assay, the test solution (containing a fixed and predetermined amount of Aβ alone, the same amount of Aβ in combination with heparan sulfate, the same amount of Aβ in combination with Al^{+++}, or the same amount of Aβ in combination with Zn^{++}) was heated for 3 hrs at 37°C (which is the physiological temperature). This heat treatment results in the formation of aggregates of Aβ. Next, the aggregates of Aβ were removed by centrifugation, and the supernatants (containing any remaining soluble Aβ) were

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[1] Solomon et al, Proc Natl Acad Sci USA 93:452-455 (1996)

incubated for 60 min with excess AMY-33 (a mouse monoclonal antibody raised against amino acids 1-28 of Aβ) to produce immunocomplexed soluble Aβ. Then, an ELISA was carried out by adding the resulting supernatant (containing the immunocomplexed Aβ) to microtiter plates that had been pre-coated with rabbit anti-Aβ antibody, resulting in the binding of any immunocomplexed Aβ in the supernatant to the plates. Next, immunocomplexed Aβ bound to the plate was measured using HRP-labeled goat anti-mouse antibody, which binds to AMY-33 of the immunocomplex, and degradation of the substrate O-phenylenediamine by the HRP was monitored by OD. As discussed below in the context of paragraph 22 of the Official action, this represents a quantitative measurement of soluble Aβ remaining after removal of the aggregate, and, by simple subtraction from the starting amount, the total amount of aggregate that was remaining.

As shown in Figure 1A of Solomon (PNAS 1996) and Figure 7A of the Solomon application:

(1) using only an aqueous solution of Aβ (1-40), the OD was about 0.1, i.e., there was not much soluble Aβ in the supernatant, and hence the conditions induced aggregation.

(2) using an aqueous solution of Aβ (1-40) containing heparan sulfate, the OD was about 0.02, i.e., not much soluble Aβ in the supernatant, and hence the conditions induced aggregation. Heparan sulfate is taught in the Solomon application and Solomon (PNAS 1996) to be associated with "aggregation of pre-existing fibrils."

(3) using an aqueous solution of Aβ (1-40) containing Al^{+++}, the OD was about 0.03, i.e., not much soluble Aβ in the supernatant, and hence the conditions induced aggregation. Al^{+++} is merely taught in the Solomon application and Solomon (PNAS 1996) to be "proposed as a 'risk factor' for Alzheimer's disease".

(4) using an aqueous solution of Aβ (1-40) containing Zn^{++}, the OD was about 0.02, i.e., not much soluble Aβ in the supernatant, and hence the conditions induced aggregation. Zn^{++} is also merely taught in the Solomon application and Solomon (PNAS 1996) to be "proposed as a 'risk factor' for Alzheimer's disease".

In a parallel set of experiments, monoclonal antibody AMY-33 was added to each sample before the first incubation, i.e., before induction of aggregation, so as to produce immunocomplexed soluble Aβ before induction of aggregation. In this manner, prevention/inhibition of aggregation was measured.

As shown in Figure 1A of Solomon (PNAS 1996) and Figure 7A of the Solomon application:

(1) using AMY-33 and only an aqueous solution of Aβ (1-40), the OD was about 0.54, i.e., there was a large amount of soluble Aβ in the supernatant, and hence a lot of prevention of aggregation.

(2) using AMY-33 and an aqueous solution of Aβ (1-40) containing heparan sulfate, the OD was about 0.65, i.e.,

there was a large amount of soluble Aβ in the supernatant, and hence a lot of prevention of aggregation.

(3) using AMY-33 and an aqueous solution of Aβ (1-40) containing Al^{+++}, the OD was about 0.04, i.e., not much soluble Aβ in the supernatant, and hence not much prevention of aggregation.

(4) using AMY-33 and an aqueous solution of Aβ(1-40) containing Zn^{++}, the OD was about 0.08, i.e., not much soluble Aβ in the supernatant, and hence not much prevention of aggregation.

Thus, the evidence in the Solomon application and in Solomon (PNAS 1996) shows that aggregation can be prevented/inhibited using AMY-33, an antibody raised against amino acids 1-28 of Aβ.

Comparable anti-aggregation experiments were carried out using monoclonal antibody 6F/3D, which was raised against amino acids 8-17 of Aβ, and whose epitope maps at amino acids 9-14 (Matsunaga et al (2002)[2]), the results of which are shown in Figure 7B of the Solomon application and Figure 1B of Solomon (PNAS 1996). As shown therein, this antibody did not significantly prevent/inhibit aggregation.

Hanan (1996)[3] confirms the results in the Solomon application and Solomon (PNAS 1996). That is, when using the same heat-induced aggregation assay and antibodies 10D5 and 6C6 (both raised against amino acids 1-28 of Aβ (Bard et al

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[2] Matsunaga et al, Biochem J 361(Pt 3):547-56 (2002)
[3] Hanan et al, Amyloid: Int J Exp Clin Invest 3:130-133 (1996)

(2003)[4]; 2H3 (raised against amino acids 1-12 of Aβ), and 1C2 (raised against amino acids 13-28 of Aβ), it was found that antibodies 10D5 and 6C6 were most effective at preventing/inhibiting the formation of aggregates (see Figure 1 thereof).

Moreover, the electron micrographs of Figure 2 of Solomon (PNAS 1996) clearly demonstrate that AMY-33 converts fibrillar Aβ to an amorphous state, and prevents/inhibits aggregation. Similarly, the electron micrographs of Figure 1 of Solomon (Fisher 1998)[5] confirm these results using 6C6 (raised against amino acids 1-28 of Aβ), i.e., this antibody also prevents/inhibits aggregation.

Solomon (PNAS 1997)[6] confirms the results in the Solomon application and Solomon (PNAS 1996). That is, when using a similar assay (but that measures disaggregation), and antibodies 6C6 (raised against amino acids 1-28 of Aβ; (Bard et al (2003)); 1C2 (raised against amino acids 13-28 of Aβ), and 14C2 (raised against amino acids 33-40 of Aβ), it was found that antibody 6C6 was most effective at solubilizing Aβ (see Figure 1 thereof).

In paragraph 21 of the Official action, the examiner notes that 6F/3D showed no discernable effect on prevention of Aβ aggregates.

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[4] Bard et al, Proc Natl Acad Sci USA, 100:2023-2028 (2003)
[5] Solomon et al in Progress in Alzheimer's and Parkinson's Diseases, edited by Fisher et al, Plenum Press, New York, 205-209 (1998)
[6] Solomon et al, Proc Natl Acad Sci USA 94:4109-4112 (1997)

However, the 6F/3D antibody does not fall within the scope of the claims because it does not inhibit aggregation. The claims all require that the antibody inhibit aggregation. Thus, it would not be expected to have any discernable effect on prevention of Aβ aggregates.

Also, in paragraph 21 of the Official action, the examiner notes that AMY-33 did not show an inhibitory effect on metal Al- or Zn-induced aggregation. The examiner contends that since Al and Zn are present in physiological conditions, these results cast doubt on the *in vivo* utility of AMY-33.

The assay in Example 2 is discussed in detail above. With this better understanding of the assay it can be seen that it is not accurate to refer to "metal-induced" beta-amyloid aggregation. In fact, aggregation of beta-amyloid in the assay was induced using heat, i.e., 37°C. The assay was carried out under three conditions, (a) heat alone, (b) heat in the presence of Zn^{++} and (c) heat in the presence of Al^{+++}. The assay does not employ "metal-induced" aggregation *per se* as apparently contended by the examiner.

It should be understood that Zn^{++} is merely one of many factors that are "speculated" in the present application as a risk factor for Alzheimer's disease. Recent evidence has suggested that 100 μM Zn^{++} actually has a protective effect against Aβ toxicity (Yoshiike et al (2001)[7]).

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[7] Yoshiike et al, J Biol Chem 276:32293-32299 (2001)

Furthermore, Al^{+++} is another factor that is merely "speculated" in the present Application as a risk factor for Alzheimer's disease. Indeed, Al^{+++} has no known physiological function (Trombley (1998)[8]).

The data in the present Application with respect to the contribution of Zn^{++} and Al^{+++} is simply inconclusive. Thus, contrary to the examiner's contention, the assay results in the presence of Al^{+++} and Zn^{++} do not cast doubt on the *in vivo* utility of AMY-33, whose results in the heat-induction assay are clear, and supported by subsequent *in vitro* and *in vivo* tests.

The examiner's attention is also invited to the attached declaration of Prof. Beka Solomon, reporting on an experiment that was conducted to show the correlation between positive results in the heat-induced aggregation assay, in the absence of Zn or Al, with positive *in vivo* results. Prof. Solomon reports on a repetition of the experiment in example 2 of the present specification, using the AMY-33 antibody as well as the 10D5 antibody. The results show that antibody 10D5 is effective in inhibiting heat-induced aggregation in the absence of Zn and Al, but it is not very effective in inhibiting heat-induced aggregation in the presence of Zn or Al. In this regard, the results are similar to the results shown with the AMY-33 antibody. The results for the AMY-33 antibody are consistent with the results reported in the

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[8] Trombley, J Neurophysiol 80:755-761 (1998)

specification of the reissue application. Both AMY-33 and 10D5 are monoclonal antibodies raised using amino acids 1-28 of β-amyloid as an immunogen. Both have been shown to maintain the solubility of soluble β-amyloid.

10D5 antibody has been shown to reduce pathology in a mouse model of Alzheimer's disease, and to cause clearance of plaques *in vivo* in a mouse model of Alzheimer's disease. It has also been reported to be effective at suppressing Aβ deposition and to act as an Aβ sink *in vivo* (see DeMattos et al (2001)[9]).

This declaration establishes that the results of the heat-induced aggregation assay in the absence of Zn and Al are the most relevant to predicting *in vivo* activity. Accordingly, it would be expected that additional antibodies, which are raised using amino acids 1-28 of β-amyloid as the immunogen, or which otherwise recognize an epitope within residues 1-28 of β-amyloid, and which inhibit heat-induced aggregation in the absence of Zn and Al, as set forth in the above-identified reissue application, would be active *in vivo* notwithstanding the results of the heat-induced aggregation assay in the presence of Zn or Al.

The examiner's attention is also directed to Figure 3 of Solomon (PNAS 1996), and Figure 2 of Solomon (Fisher 1998), and Figure 2 of Solomon (PNAS 1997). These experiments confirm the above-discussed results.

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[9] DeMattos et al, Proc Nat Acad Sci USA 98:8850-8855 (2001)

Figure 3 of Solomon (PNAS 1996) shows that soluble Aβ has no effect on the florescence of the dye Thioflavin T, whereas aggregated Aβ changes the excitation spectrum of Thioflavin T. Adding AMY-33 to soluble Aβ, prior to aggregation (incubation at 37°C), prevented the change in florescence, i.e., prevented/inhibited aggregation.

Figure 2 of Solomon (Fisher 1998) shows that soluble Aβ has no effect on the florescence of the dye Thioflavin T, whereas aggregated Aβ changes the excitation spectrum of Thioflavin T. Adding 6C6, 10D5, 2H3, 1C2, or 266 to soluble Aβ, prior to aggregation (incubation at 37°C), prevented the change in florescence, i.e., prevented/inhibited aggregation.

Figure 2 of Solomon (PNAS 1997) shows that anti- Aβ antibodies disrupt Aβ fibrils. Fibrils of Aβ were first formed, and then incubated with 6C6 or IC2. 6C6 was found to extensively disrupt fibrils, whereas IC2 was found to only slightly interfere with fibril disaggregation.

In paragraph 22 of the Official action, the examiner contends that the assays preformed measure A495 (OD) or fluorescence, which are relative and not quantitative measurements.

Contrary to the examiner's contention, Hanan (1996) shows that the OD data was concentration dependent (see Figure 1, insert), and thus a quantitative measurement. Further, the fluorescence was concentration dependent (see e.g., Figure 2 of Solomon (Fisher 1998) and the legend of Figure 2 of Solomon (PNAS 1997)), and thus a quantitative measurement.

In paragraph 23 of the Official action, the examiner contends that the specification and the prior art do not provide any support to correlate the prevention, disaggregation or inhibition of aggregation with an alleviation of symptoms or providing some relief to the patient.

The examiner is requested to note that the PDAPP mouse has been recognized in the art as being a major breakthrough in the production of an animal model for Alzheimer's disease. The importance and breakthrough nature of the PDAPP mouse is evident, i.e., it was a cover story in *Nature* in 1995 (Games et al (1995)[10]). The PDAPP transgenic mouse described in Games et al (1995) exhibit age- and brain region-dependent development of typical amyloid plaques, dystrophic neurites, loss of presynaptic terminals, astrocytosis and microgliosis. These lesions in the PDAPP mouse brain tissue are typical of many of the neuropathological hallmarks associated with Alzheimer's disease. Games et al (1995) also teaches that in the PDAPP mice, neurodegeneration and inflammation characteristic of Alzheimer's disease, with associated Aβ plaque deposition and certain regions of afflicted brain parenchyma, are present. Deposition of brain deposits in the PDAPP mice increases with age, as is found in Alzheimer's disease. Thus, the PDAPP

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[10] Games et al, Nature, 373:523-527 (1995)

mouse shows much of the pathology seen in Alzheimer's disease patients.

Games et al (1995) concludes, at page 527, second paragraph, first column:

> A most notable feature of these transgenic mice is their Alzheimer-like neuropathology .... Our transgenic model ... offers a means to test whether compounds that lower Aβ production and/or reduce its neurotoxicity *in vitro* can produce beneficial effects in an animal model prior to advancing such drugs into human clinical trials.

Similarly, Schenk et al (1999),[11] which was a cover story in *Nature* in 1999, concludes, at page 177, paragraph bridging columns 1 and 2:

> To our knowledge, this is the first report of a clinically relevant treatment that reduces the progression of AD-like neuropathology in a transgenic model [the PDAPP mouse] of the disease .... Collectively, the results suggest that amyloid β immunization may prove beneficial for both the treatment and prevention of Alzheimer's disease.

Thus, Games et al (1995) and Schenk et al (1999) teach that the PDAPP mouse exhibits many of the pathological characteristics of Alzheimer's disease, and is regarded in the art as a model reasonably predictive of results in humans.

As shown in Bard et al (2003); Bard et al (2000);[12] and Bacskai et al (2001),[13] *inter alia*, antibodies 6C6 and 10D5 (again both raised against amino acids 1-28 of Aβ) were

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[11] Schenk et al, Nature 400:173-177 (1999)
[12] Bard et al, Nature Medicine 6:916-919 (2000)
[13] Bacskai et al, Nature Medicine 7:369-372 (2001)

effective in clearing Aβ plaques in *in vivo* and *ex vivo* experiments with PDAPP mice.

In paragraph 24 of the Official action, the examiner contends that the specification does not provide sufficient guidance that would enable the skilled artisan to conceive of and make any antibody that would prevent or reduce aggregation or disaggregate aggregates in a subject.

In view of the amendments to the claims (new claims 150-167), which recite that the epitope is within amino acids 1-28 of Aβ, or is obtainable using 1-28 of Aβ as the immunogen, applicant respectfully submits that the examiner's rejection has been rendered moot.

In paragraph 25 of the Official action, the examiner contends that undue trial and error experimentation would be required to make antibodies that are capable of prevention or reduction of Aβ aggregates or disaggregate the same in patients.

Contrary to the examiner's contention, as discussed above, the present specification shows that AMY-33 (raised against Aβ amino acids 1-28) inhibits aggregation of Aβ, Hanan et al (1996) shows that 6C6 and 10D5 (both raised against amino acids 1-28) inhibit aggregation of Aβ, and Solomon (PNAS 1997) shows that 6C6 (raised against amino acids 1-28) causes disaggregation of Aβ aggregates. On the other hand, the evidence shows that 6F/3D (raised against Aβ amino acids 8-17) does not inhibit Aβ aggregation. It would clearly <u>not</u> require undue experimentation for one skilled in the art to produce

antibodies with the claimed specificity (which the post-filing evidence (Bard et al (2000); Bard et al (2003); Bacskai et al (2001); and DeMattos et al (2001)) clearly demonstrates are effective at inhibiting aggregation of Aβ and disaggregating Aβ aggregates). That is, one could merely use, e.g., Aβ 1-28 as an immunogen, and assay for inhibition of aggregation or disaggregation, as described in the present application.

In paragraph 27 of the Official action, the examiner cites Walker et al for teaching that the anti-Aβ antibody 10D5 did not disaggregate, prevent or inhibit aggregation.

Applicant respectfully submits that the examiner has mischaracterized Walker et al. Walker et al merely relates to *in vivo* imaging of Aβ deposits in the brain. Walker et al did not look for, much less carry out any experiments to measure disaggregation or prevention/inhibition of aggregation. In any event, as discussed above, Hanan et al (1996), Solomon (Fisher 1998), and the attached Solomon declaration, clearly show that 10D5 inhibited aggregation of Aβ.

In paragraph 28 of the Official action, the examiner cites Pan et al for teaching that anti-Aβ antibodies, i.e., 3D6, decreases plaques in PDAPP mice by decreasing the concentration of Aβ in the central nervous system, not by disaggregation. Thus, the examiner contends that Pan et al teaches that Aβ plaques are not disassembled or prevented *per se*, but their formation is inhibited or in another sense slowed.

First of all, the experimentation reported in Pan was conducted on normal ICR mice, and not the PDAPP Alzheimer's disease mouse model. Thus, these mice do not spontaneously form amyloid plaques in the absence of antibody. The fact that the antibodies were shown to decrease the influx of Aβ into the brain does not necessarily mean that plaque is decreased.

Pan et al provides evidence that 3D6 can reduce the blood-to-brain influx of Aβ. However, this is merely one possible mechanism of action of 3D6. Pan et al does not exclude other mechanisms of action of 3D6. Indeed other mechanisms of action were not even tested in Pan et al. In this regard, Pan et al teaches, at page 614:

> Thus, we have shown that peripherally administered antibodies can decrease the availability of blood-borne Aβ to the brain. This does not rule out other routes of action, such as direct penetration of the antibody into the CNS or an influence on the solubility and CSF dynamics of Aβ ... In addition the N-terminal epitope (1-28) of Aβ is essential for aggregation (21) and the 3-6 sequential epitope is particularly important (8, 9). mAb3D6 is directed to the 1-5 sequence and likely prevented the aggregation of Aβ. (Emphases added)

Hence, contrary to the examiner's contention, Pan et al presents no experimental evidence on the issue of plaque disassembly or prevention, although the above quote does not rule out the possibility of such routes of action, i.e., disassembly or prevention of plaque.

In this regard, the examiner is requested to note that DeMattos et al (2001) states that 10D5 and 3D6, which are

effective at suppressing Aβ deposition *in vivo* in PDAPP mice are also able to decrease the concentration of Aβ in the central nervous system, i.e., act as Aβ sinks. Thus, this reference concludes that disaggregation is one mechanism of inhibition of Aβ aggregation that contributes to the effects of peripherally administered anti-amyloid antibodies, and that they can not exclude the possibility that antibodies, such as 266, enter the brain and sequester a soluble, toxic Aβ species.

In any event, the claims have been amended (new claims 150-167) to recite "inhibition" of aggregation, thereby rendering moot this aspect of the examiner's rejection.

In paragraph 29 of the Official action, the examiner cites Akiyama et al for teaching that 6F/3D does not readily bind plaques in cerebral cortex sample from an Alzheimer's patient.

However, this result is entirely consistent with the data and teachings in the Solomon application and Solomon (PNAS 1996), which shows that 6F/3D does not prevent aggregation, and teaches that 6F/3D does not bind to a disaggregation epitope. Applicant respectfully draws the examiner's attention to the fact that 6F/3D tests negative in the Solomon experiments and is, therefore, not covered by the claims. Note, Akiyama et al teaches, at page 328, right-hand column, that extracellular deposits retain immunoreactivity of N-terminal residues.

In paragraph 30 of the Official action, the examiner cites Perutz et al as showing the structure of amyloid fibers, and as providing the basis for the examiner's belief that anti-Aβ antibodies may inhibit or slow aggregation, but do not disassemble aggregates.

As discussed above, the data in Solomon (PNAS 1997) clearly demonstrate disaggregation of Aβ aggregates.

In any event, in view of the amendments to the claims (new claims 150-167) that recite "inhibition" of aggregation, applicant respectfully submits that the examiner's rejection has been rendered moot.

For all of these reasons, reconsideration and withdrawal of this rejection is respectfully urged.

The present specification has now been amended to correct an obvious error in the first paragraph of column 7. The patent stated that in a preferred embodiment the expression vector includes the sequence for a human monoclonal antibody "that is an anti-β-amyloid monoclonal antibody with heparan-like characteristics." The reference to "heparan-like characteristics" is nonsensical. The only reference to heparan in the specification is as an aggregating agent (column 11, lines 27-29, and column 16, lines 9-12). The antibodies inhibit aggregation of β-amyloid in the presence or absence of heparan sulfate. Thus, the antibodies do not have "heparan-like characteristics." To correct this obvious error, the words "with heparan-like characteristics" have now been deleted from this paragraph.

It should further be noted that column 16, lines 5-9, of the patent state:

> Binding of mAb AMY-33 to βA4 prevents self-aggregation of the β-amyloid, probably by recognizing the sequence 25-28 located in the proposed aggregation fragment comprising the amino acids between 25-28 (Yankher et al., 1990)(FIG.8).

It is not presently believed that the epitope of AMY-33 is the sequence 25-28 of β-amyloid. However, the above quote only indicates that it "probably" recognizes this sequence. Therefore, there is no necessity to correct it. The present statement, however, clarifies the record in this regard.

Copies of all publications cited herein that are not already of record or attached to the Solomon declaration are attached hereto.

It is submitted that all of the claims now present in the case clearly define the references of record. Reconsideration and allowance are therefore earnestly solicited.

Respectfully submitted,

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CLAIM SUPPORT CHART

CLAIM	SUPPORT
Claim 150. A **pharmaceutical formulation**, comprising:	**C. 9, L. 23-25**: It is preferable to present it as a **pharmaceutical formulation.** The **formulations** of the present inventions comprise….
(A) an **antibody** or **antigen binding fragment** thereof, wherein:	**C. 5, L. 30-33**: The **antibodies**, or peptide mimicking the binding site, must bind to an epitope on the target molecule which is a region responsible for folding or aggregation. **C. 9, L. 24-26**: The formulations of the present invention comprise … **the monoclonal antibody** …. **C. 9, L. 45-48**: [T]he use of engineered **monoclonal antibodies and their fragments** … can be used in the present invention. **C. 12, L. 1-8**: Alternatively, commercially available **antibodies** can be used….A **polyclonal**, affinity purified rabbit IgG obtained against the synthetic Alzheimer β-amyloid. **C. 16, L. 26-31**: Recent advances in antibody engineering technology, as well as in the development of suitable delivery systems…make it possible to develop **functional** small **antibody fragments** to serve as therapeutic chaperones for the treatment of Alzheimer's disease….
(i) said antibody and said fragment **recognize an epitope within residues 1-28 of beta-amyloid**, and	**C. 5, L. 30-33**: The antibodies, or peptide mimicking the binding site, must bind to an **epitope** on the target molecule **which is**

CLAIM	SUPPORT
	a region responsible for folding or aggregation. **C. 6, L. 23-27:** In a further preferred embodiment the monoclonal antibody is an anti-β-amyloid and is designated AMY-33 which **recognizes amino acids 1-28 of β-amyloid.** **C. 15, L. 35-38:** mAb AMY-33…**raised against peptide[s]** …**1-28**…of the **β-amyloid.** **C. 15, L. 43-46:** The antibody AMY-33, which is supposed to recognize **an epitope spanned between sequence 1-28,** inhibits the β-amyloid aggregation
(ii) said antibody and said fragment **inhibit aggregation of beta-amyloid;** and	**C. 6, L. 21-23:** In the preferred embodiment the human monoclonal antibody that binds to an aggregating protein and **which prevents aggregation** is utilized. **C. 9, L. 61-62: The antibodies effect on the inhibition of aggregation ….** **C. 15, L. 43-46:** The antibody AMY-33, which is supposed to recognize an epitope spanned between sequence 1-28, **inhibits the β-amyloid aggregation….**
(B) a **pharmaceutically acceptable carrier.**	**C. 9, L. 24-27:** The formulations of the present invention comprise at least one active ingredient: the monoclonal antibody or expression vector together with one or more **pharmaceutically acceptable carriers….**
Claim 151. The pharmaceutical formulation of claim 150,	See claim 150
wherein said antibody is a **monoclonal antibody.**	**C. 5, L. 51-53:** In the preferred embodiment of the

CLAIM	SUPPORT
	method, the target molecule is β-amyloid and the **monoclonal antibody** is an anti-β-amyloid **monoclonal.** **C. 6, L. 1-6:** Once an appropriate **monoclonal antibody** with chaperone-like activity is found or engineered…, the present invention provides for its use therapeutically to prevent or reduce protein aggregation in vivo. **C. 9, L. 22-28:** It is preferable to present it as a pharmaceutical formulation. The formulations of the present invention comprise at least one active ingredient: **the monoclonal antibody** or expression vector together with one or more pharmaceutical acceptable carriers and optionally other therapeutic ingredients.

Claim 152. The pharmaceutical formulation of claim 151,	See claim 151
wherein said antibody is a **human monoclonal antibody.**	**C. 6, L. 21-23:** In the preferred embodiment the **human monoclonal antibody** that binds to an aggregating protein and which prevents aggregation is utilized. **C. 7, L. 7-12:** In a preferred embodiment the expression vector includes the sequence for a **human monoclonal antibody** that is an anti-β-amyloid monoclonal antibody with heparin-like characteristics.

Claim 153. The pharmaceutical formulation	See claim 151

CLAIM	SUPPORT
of claim 151,	
wherein said antibody is a **genetically-engineered monoclonal antibody.**	**C. 9, L. 45-48:** the use of **engineered monoclonal antibodies** and their fragments, as well as peptides which mimic the binding site for the antigen on the antibody can be used in the present invention. **C. 10, L. 1-5:** The present invention uses **genetically engineered antibodies** obtained from such selected antibodies as protecting agents of in vivo aggregation of their antigen….

Claim 154. The pharmaceutical formulation of claim 153,	See claim 153
wherein said antibody is a **single-chain antibody.**	**C. 6. L. 27-29:** Work by Duenas et al. (1994) and Marasco et al. (1993) have shown that **single chain monoclonal antibodies** are efficient for intracellular expression in eukaryotes. **C. 7, L. 9-11:** In a further preferred embodiment, the expression vector includes the sequence for the **single chain monoclonal antibody** of the above anti-β-amyloid mAb. **C. 16, L. 34-37:** Application of the above findings for in vivo aggregation, can confer to **single chain antibodies** or other engineered antibody fragments, a protective role in the renaturation of recombinant proteins

Claim 155. The pharmaceutical formulation of any one of claims 150-154,	See claims 150-154

CLAIM	SUPPORT
wherein said beta-amyloid is **human beta-amyloid.**	**C. 8, L. 19-21**: The expression vector containing the sequence for the anti-aggregation molecule may be administered to mammals, including **humans…** **C. 11, L. 20-23**: Amyloid peptides, Aβ 1-40 (Cat. No. A-5813) and Aβ 1-28 (Cat. No. A-1084) corresponding to amino acids 1-40 and 1-28 of Aβ respectively, were produced from Sigma Chemical Co., St. Louis, MO., USA. **C. 12, L. 1-3**: Alternatively, commercially available antibodies can be used. **α-Human β-amyloid** 6F/3D was obtained…. **C. 16, L. 27-33**: Recent advances in antibody engineering technology, as well as in the development of suitable delivery systems…make it possible to develop functional small antibody fragments to serve as therapeutic chaperones for the treatment of **Alzheimer's disease as well as other human amyloidosis diseases….**

Claim 156. A pharmaceutical formulation, comprising:	**C. 9, L. 23-25**: It is preferable to present it as a **pharmaceutical formulation.** The **formulations** of the present inventions comprise….
(A) an **antibody** or **antigen binding fragment** thereof, wherein:	**C. 5, L. 30-33**: The **antibodies**, or peptide mimicking the binding site, must bind to an epitope on the target molecule which is a region responsible for folding or aggregation. **C. 9, L. 24-26**: The formulations of the present invention comprise … **the**

CLAIM	SUPPORT
	monoclonal antibody …. **C. 9, L. 45-48**: [T]he use of engineered **monoclonal antibodies and their fragments** … can be used in the present invention. **C. 12, L. 1-8**: Alternatively, commercially available **antibodies** can be used….A **polyclonal**, affinity purified rabbit IgG obtained against the synthetic Alzheimer β-amyloid. **C. 16, L. 26-31**: Recent advances in antibody engineering technology, as well as in the development of suitable delivery systems…make it possible to develop **functional** small **antibody fragments** to serve as therapeutic chaperones for the treatment of Alzheimer's disease….
(i) said antibody is **obtainable using residues 1-28 of beta-amyloid as an immunogen**, and	**C. 6, L. 23-27:** In a further preferred embodiment the monoclonal antibody is an anti-β-amyloid and is designated AMY-33 **which recognizes amino acids 1-28 of β-amyloid.** **C. 15, L. 35-38:** mAb AMY-33…**raised against peptide[s]** …**1-28**…of the β-amyloid. **C. 15, L. 43-46:** The antibody AMY-33, which is supposed to **recognize an epitope spanned between sequence 1-28,** inhibits the β-amyloid aggregation….
(ii) said antibody and said fragment **inhibit aggregation of beta-amyloid**; and	**C. 6, L. 21-23:** In the preferred embodiment the human monoclonal antibody that binds to an aggregating protein and **which prevents aggregation** is utilized. **C. 9, L. 61-62: The**

CLAIM	SUPPORT
	antibodies effect on the inhibition of aggregation …. **C. 15, L. 43-46:** The antibody AMY-33, which is supposed to recognize an epitope spanned between sequence 1-28, **inhibits the β-amyloid aggregation….**
(B) a **pharmaceutically acceptable carrier.**	**C. 9, L. 24-27:** The formulations of the present invention comprise at least one active ingredient: the monoclonal antibody or expression vector together with one or more **pharmaceutically acceptable carriers….**

Claim 157. The pharmaceutical formulation of claim 156,	See claim 156
wherein said antibody is a **monoclonal antibody.**	**C. 5, L. 51-53:** In the preferred embodiment of the method, the target molecule is β-amyloid and the **monoclonal antibody** is an anti-β-amyloid **monoclonal.** **C. 6, L. 1-6:** Once an appropriate **monoclonal antibody** with chaperone-like activity is found or engineered…, the present invention provides for its use therapeutically to prevent or reduce protein aggregation in vivo. **C. 9, L. 22-28:** It is preferable to present it as a pharmaceutical formulation. The formulations of the present invention comprise at least one active ingredient: **the monoclonal antibody** or expression vector together with one or more pharmaceutical acceptable carriers and optionally other

CLAIM	SUPPORT
	therapeutic ingredients.

Claim 158. The pharmaceutical formulation of claim 157,	See claim 157
wherein said antibody is a **human monoclonal antibody.**	**C. 6, L. 21-23:** In the preferred embodiment the **human monoclonal antibody** that binds to an aggregating protein and which prevents aggregation is utilized. **C. 7, L. 7-12:** In a preferred embodiment the expression vector includes the sequence for a **human monoclonal antibody** that is an anti-β-amyloid monoclonal antibody with heparin-like characteristics.

Claim 159. The pharmaceutical formulation of claim 157,	See claim 157
wherein said antibody is a **genetically-engineered monoclonal antibody.**	**C. 9, L. 45-48:** the use of **engineered monoclonal antibodies** and their fragments, as well as peptides which mimic the binding site for the antigen on the antibody can be used in the present invention. **C. 10, L. 1-5:** The present invention uses **genetically engineered antibodies** obtained from such selected antibodies as protecting agents of in vivo aggregation of their antigen….

Claim 160. The pharmaceutical formulation of claim 159,	See claim 159
wherein said antibody is a **single-chain antibody.**	**C. 6. L. 27-29:** Work by Duenas et al. (1994) and Marasco et al. (1993) have shown that **single chain monoclonal antibodies** are

CLAIM	SUPPORT
	efficient for intracellular expression in eukaryotes. **C. 7, L. 9-11:** In a further preferred embodiment, the expression vector includes the sequence for the **single chain monoclonal antibody** of the above anti-β-amyloid mAb. **C. 16, L. 34-37:** Application of the above findings for in vivo aggregation, can confer to **single chain antibodies** or other engineered antibody fragments, a protective role in the renaturation of recombinant proteins

Claim 161. The pharmaceutical formulation of any one of claims 156-160,	See claims 156-160
wherein said beta-amyloid is **human beta-amyloid.**	**C. 8, L. 19-21**: The expression vector containing the sequence for the anti-aggregation molecule may be administered to mammals, including **humans…** **C. 11, L. 20-23**: Amyloid peptides, Aβ 1-40 (Cat. No. A-5813) and Aβ 1-28 (Cat. No. A-1084) corresponding to amino acids 1-40 and 1-28 of Aβ respectively, were produced from Sigma Chemical Co., St. Louis, MO., USA. **C. 12, L. 1-3**: Alternatively, commercially available antibodies can be used. **α-Human β-amyloid** 6F/3D was obtained…. **C. 16, L. 27-33**: Recent advances in antibody engineering technology, as well as in the development of suitable delivery systems…make it possible to develop functional small

CLAIM	SUPPORT
	antibody fragments to serve as therapeutic chaperones for the treatment of **Alzheimer's disease as well as other human amyloidosis diseases**….

Claim 162. A pharmaceutical formulation, comprising:	**C. 9, L. 23-25**: It is preferable to present it as a **pharmaceutical formulation**. The **formulations** of the present inventions comprise….
(A) an **antibody** or **antigen binding fragment** thereof, wherein:	**C. 5, L. 30-33**: The **antibodies**, or peptide mimicking the binding site, must bind to an epitope on the target molecule which is a region responsible for folding or aggregation. **C. 9, L. 24-26**: The formulations of the present invention comprise … **the monoclonal antibody** …. **C. 9, L. 45-48**: [T]he use of engineered **monoclonal antibodies and their fragments** … can be used in the present invention. **C. 12, L. 1-8**: Alternatively, commercially available **antibodies** can be used….A **polyclonal**, affinity purified rabbit IgG obtained against the synthetic Alzheimer β-amyloid. **C. 16, L. 26-31**: Recent advances in antibody engineering technology, as well as in the development of suitable delivery systems…make it possible to develop **functional** small **antibody fragments** to serve as therapeutic chaperones for the treatment of Alzheimer's disease….
(i) said antibody and said fragment **recognize an**	**C. 5, L. 30-33**: The antibodies, or peptide

CLAIM	SUPPORT
epitope within residues 1-28 of beta-amyloid, and	mimicking the binding site, must bind to an **epitope** on the target molecule **which is a region responsible for folding or aggregation.** **C. 6, L. 23-27:** In a further preferred embodiment the monoclonal antibody is an anti-β-amyloid and is designated AMY-33 which **recognizes amino acids 1-28 of β-amyloid.** **C. 15, L. 35-38:** mAb AMY-33…**raised against peptide[s] …1-28…**of the **β-amyloid.** **C. 15, L. 43-46:** The antibody AMY-33, which is supposed to recognize **an epitope spanned between sequence 1-28,** inhibits the β-amyloid aggregation
(ii) said antibody and said fragment **maintain the solubility of soluble beta-amyloid**; and	**C. 1., L. 35-37**: In vitro **aggregation limits** the protein stability, **solubility** and yields in production of recombinant proteins. **C. 3, L. 54-56**: which **prevents aggregation** and allows biological activity of the target molecule. **C. 6, L. 12-15**: …binds to an aggregating protein which is the cause of a disease and which **prevents aggregation** and yet allows the protein to be bioactive. **Col. 10, L. 1-5**: The present invention uses genetically engineered antibodies obtained from such selected antibodies of in vivo aggregation of their antigen, leading to production of a **soluble and stabilized protein.** **Col. 10, L. 16-19**: The identification of such

CLAIM	SUPPORT
	classes of sequences that play a role in the folding-unfolding and/or **solubilization**-aggregation provides the basis of the present invention for prevention of aggregation. **C. 13, L. 30-32, 38-40, Fig. 7a and 7b:** The residual **soluble β-amyloid** was incubated for another one hour at 37° C with mAbs AMY-33 and/or 6F3D at equal molar ratio antibody/antigen….**The amount of mAb bound will be proportional to the amount of soluble amyloid which remained after exposure to aggregating conditions.**
(B) a **pharmaceutically acceptable carrier.**	**C. 9, L. 24-27:** The formulations of the present invention comprise at least one active ingredient: the monoclonal antibody or expression vector together with one or more **pharmaceutically acceptable carriers….**

Claim 163. The pharmaceutical formulation of claim 162,	See claim 162
wherein said antibody is a **monoclonal antibody.**	**C. 5, L. 51-53:** In the preferred embodiment of the method, the target molecule is β-amyloid and the **monoclonal antibody** is an anti-β-amyloid **monoclonal. C. 6, L. 1-6:** Once an appropriate **monoclonal antibody** with chaperone-like activity is found or engineered…, the present invention provides for its use therapeutically to prevent or reduce protein aggregation in vivo.

CLAIM	SUPPORT
	C. 9, L. 22-28: It is preferable to present it as a pharmaceutical formulation. The formulations of the present invention comprise at least one active ingredient: **the monoclonal antibody** or expression vector together with one or more pharmaceutical acceptable carriers and optionally other therapeutic ingredients.

Claim 164. The pharmaceutical formulation of claim 163,	See claim 163
wherein said antibody is a **human monoclonal antibody.**	**C. 6, L. 21-23:** In the preferred embodiment the **human monoclonal antibody** that binds to an aggregating protein and which prevents aggregation is utilized. **C. 7, L. 7-12:** In a preferred embodiment the expression vector includes the sequence for a **human monoclonal antibody** that is an anti-β-amyloid monoclonal antibody with heparin-like characteristics.

Claim 165. The pharmaceutical formulation of claim 163,	See claim 163
wherein said antibody is a **genetically-engineered monoclonal antibody.**	**C. 9, L. 45-48:** the use of **engineered monoclonal antibodies** and their fragments, as well as peptides which mimic the binding site for the antigen on the antibody can be used in the present invention. **C. 10, L. 1-5:** The present invention uses **genetically engineered antibodies** obtained from such selected

CLAIM	SUPPORT
	antibodies as protecting agents of in vivo aggregation of their antigen….

Claim 166. The pharmaceutical formulation of claim 165,	See claim 165
wherein said antibody is a **single-chain antibody.**	**C. 6. L. 27-29:** Work by Duenas et al. (1994) and Marasco et al. (1993) have shown that **single chain monoclonal antibodies** are efficient for intracellular expression in eukaryotes. **C. 7, L. 9-11:** In a further preferred embodiment, the expression vector includes the sequence for the **single chain monoclonal antibody** of the above anti-β-amyloid mAb. **C. 16, L. 34-37:** Application of the above findings for in vivo aggregation, can confer to **single chain antibodies** or other engineered antibody fragments, a protective role in the renaturation of recombinant proteins

Claim 167. The pharmaceutical formulation of any one of claims 162-166,	See claims 162-166
wherein said beta-amyloid is **human beta-amyloid.**	**C. 8, L. 19-21:** The expression vector containing the sequence for the anti-aggregation molecule may be administered to mammals, including **humans…** **C. 11, L. 20-23:** Amyloid peptides, Aβ 1-40 (Cat. No. A-5813) and Aβ 1-28 (Cat. No. A-1084) corresponding to amino acids 1-40 and 1-28 of Aβ respectively, were produced from Sigma Chemical

CLAIM	SUPPORT
	Co., St. Louis, MO., USA. **C. 12, L. 1-3**: Alternatively, commercially available antibodies can be used. **α-Human β-amyloid** 6F/3D was obtained…. **C. 16, L. 27-33**: Recent advances in antibody engineering technology, as well as in the development of suitable delivery systems…make it possible to develop functional small antibody fragments to serve as therapeutic chaperones for the treatment of **Alzheimer's disease as well as other human amyloidosis diseases….**

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